CSIRO PUBLISHING
www.publish.csiro.au/journals/is *Invertebrate Systematics*, 2005, **19**, 531–539

Phylogenetic relationships of the Australian Leptophlebiidae (Ephemeroptera)

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Abstract. Phylogenetic relationships among the Australian Leptophlebiidae genera and selected genera from South America and New Zealand were investigated using a cladistic analysis of 43 morphological characters. The outcomes of this analysis were largely consistent with the higher-level relationships previously proposed by Pescador and Peters (1980). The monophyly of the *Meridialaris* lineage (comprising *Austrophlebioides*, *Tillyardophlebia*, *Kirrara*, 'WT sp. 1' and 'WT sp. 2' from Australia, *Meridialaris* from South America and *Deleatidium* and *Atalophlebioides* from New Zealand) was strongly supported, as was the monophyly of the *Nousia* lineage (*Nyungara*, *Nousia* and *Koorrnonga*). However, Australian genera assigned to the *Hapsiphlebia* lineage (*Atalophlebia*, *Kalbaybaria, Ulmerophlebia*, *Jappa* and *Atalomicria*) did not form a monophlyletic group. There was support for a sister-group relationship between the *Dactylophlebia* and *Meridialaris* lineages, and for the placement of *Garinjuga* (*Penaphlebia* lineage) as the sister-group to a large clade comprising genera of the *Nousia*, *Dactylophlebia* and *Meridialaris* lineages. The phylogenetic analysis provided some clarification of the affinities of *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga.* These genera appear to belong to lineages not recognised previously among the Gondwanan Leptophlebiidae.

Introduction

The Leptophlebiidae is the most speciose mayfly family in Australia, with 69 described species and 17 genera. All Australian genera belong to the subfamily Atalophlebiinae and, with the exception of the genus *Nousia*, which occurs in Australia and South America, are endemic to Australia. The phylogenetic affinities of the Australian leptophlebiid genera are believed to be with genera present on other southern landmasses, particularly southern South America and New Zealand, which were once part of the ancient continent of Gondwana (Peters and Edmunds 1972; Tsui and Peters 1975; Pescador and Peters 1980; Towns and Peters 1980, 1996). Pescador and Peters (1980) proposed a higher-level phylogeny for this Gondwanan group of genera, in which they recognised five major lineages: the *Hapsiphlebia, Penaphlebia*, *Nousia*, *Dactylophlebia* and *Meridialaris* lineages (Fig. 1). Their phylogeny was based on morphological characters of nymphs and adults and was constructed using traditional Hennigian methods. Character polarity was inferred by reference to a hypothetical ancestor and the possession of shared derived character states (synapomorphies) was used to define each lineage. The majority of taxa in the analysis were from southern South America, with additional genera (known at the time) from Australia, New Zealand and southern Africa. Phylogenetic relationships among the New Zealand genera were investigated by Towns and Peters (1980) and again more recently by Towns and Peters (1996).

Derived character states identified by Pescador and Peters (1980) and Towns and Peters (1980, 1996) for each of the five lineages have been used to determine the phylogenetic affinities of the Australian genera. The genera *Atalophlebia*, *Kalbaybaria, Ulmerophlebia*, *Jappa* and *Atalomicria* have been assigned to the *Hapsiphlebia* lineage (Pescador and Peters 1980; Campbell 1993). *Garinjuga* is presently the only Australian member of the *Penaphlebia* lineage (Campbell and Suter 1988). *Nousia*, *Koorrnonga* and *Nyungara* have been placed in the *Nousia* lineage (Pescador and Peters 1980; Dean 1987; Campbell and Suter 1988). *Austrophlebioides*, *Kirrara*, *Tillyardophlebia* and several undescribed genera are believed to belong to the *Meridialaris* lineage (Pescador and Peters 1980; Campbell and Peters 1986; Campbell and Suter 1988; Dean 1997). The *Dactylophlebia* lineage is not known from Australia. Although most Australian genera can be assigned to one of the five lineages, the affinities of several genera, including *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga*, are unclear (Dean 1987, 1988, 2000; Dean *et al*. 1999). These genera cannot be placed in any of the five lineages as presently defined.

Recently, Christidis (2001) presented the first cladistic analysis of the Australian Leptophlebiidae, based on morphological data. Although the main focus of that study was on phylogenetic relationships of Australian genera and species of the *Meridialaris* lineage, the study also provided a preliminary assessment of the higher-level phylogeny

10.1071/IS05022 1445-5226/05/060531

proposed by Pescador and Peters (1980). The cladistic analysis of the Australian leptophlebiid genera supported the monophyly of the *Meridialaris* and *Nousia* lineages, but not the monophyly of the *Hapsiphlebia* lineage. The analysis of Christidis (2001) was unable to resolve the affinities of *Neboissophlebia*, *Bibulmena* and *Loamaggalangta*, or the phylogenetic position of *Garinjuga* (*Penaphlebia* lineage).

The analysis presented in this paper builds on the work of Christidis (2001) with the addition of new morphological characters, a newly described genus from Australia, as well as the addition of selected genera from South America and New Zealand, to further test the monophyly of the *Meridialaris* lineage and the higher-level relationships proposed by Pescador and Peters (1980).

Methods

Taxa

Representative species of sixteen of the seventeen genera described from Australia were included, along with two undescribed species, 'WT sp. 1' and 'WT sp. 2', believed to belong to two new genera (Table 1). The Australian monotypic genus *Thraulophlebia* was omitted from the present study because no specimens of the type species, *Thraulophlebia lucida* (Ulmer), could be obtained. Selected genera from New Zealand (*Atalophlebioides*, *Deleatidium*, *Austroclima* and *Mauiulus*) and South America (*Meridialaris*) were also included in the analysis to test the higher-level relationships within the group and the monophyly of the *Meridialaris* lineage.

Morphological characters

Morphological characters were obtained from examination of nymphal and adult material, and from the literature (Table 1). Nymphal characters were scored from various body regions including the mouthparts, thorax, abdomen and legs. Adult characters were scored from the hind wings, male genitalia and female abdomen. In total, 43 characters were obtained, 27 of which were binary and 16 were multistate. Characters and character states used in the analysis are listed Table 2. The data matrix is given in Table 3 and is also available as an Accessory Publication on the *Invertebrate Systematics* website. The terminology used in this study follows that of Edmunds *et al.* (1976). Morphological characters identified by other systematists as phylogenetically informative (particularly Pescador and Peters 1980; and Towns and Peters 1980, 1996) were included in the matrix. Adults and nymphs of undescribed species were associated by rearing.

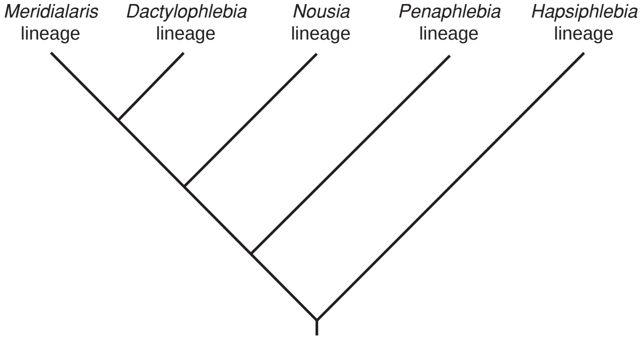


Fig. 1. Phylogeny proposed by Pescador and Peters (1980).

Phylogenetic analyses

Cladistic analyses were performed using PAUP* version 4.0b10 (Swofford 2002). The branch-and-bound search option was used, with characters treated as unordered and equally weighted. Two representative species from the subfamily Leptophlebiinae, *Leptophlebia cupida* (Say) and *Leptophlebia marginata* (Linn.), were used as the outgroup. Bootstrap analysis (1000 randomisations, full heuristic search, simple addition, tree bisection reconnection (TBR) branch swapping) was used to assess support for each node of the tree (Felsenstein 1985; Hillis and Bull 1993).

Results

Cladistic analysis resulted in two most parsimonious trees, each with a length of 113 steps, a consistency index (*CI*) of 0.5575 and a retention index (*RI*) of 0.8031. The two trees shown in Fig. 2 differ only in the placement of *Austrophlebioides, Meridialaris* and *Tillyardophlebia*. In the strict consensus tree (Fig. 3), relationships among *Austrophlebioides, Tillyardophlebia, Meridialaris* and the clade containing *Kirrara*, *Deleatidium* and *Atalophlebioides* were unresolved.

Genera of the *Hapsiphlebia* lineage, *Kalbaybaria, Atalophlebia*, *Atalomicria*, *Jappa* and *Ulmerophlebia,* did not form a monophyletic group. There was strong support for a sister-group relationship between *Jappa* and *Ulmerophlebia*, but the grouping of *Atalophlebia* and *Atalomicria* as sister-taxa was poorly supported.

Neboissophlebia, *Bibulmena*, *Loamaggalangta* and *Kaninga* were placed at the base of a well supported clade containing all ingroup genera except those of the *Hapsiphlebia* lineage. *Garinjuga* (*Penaphlebia* lineage) was placed as sister to a clade containing genera of the *Nousia*, *Dactylophlebia* and *Meridialaris* lineages, although this relationship was poorly supported. *Nousia*, *Koorrnonga* and *Nyungara* (*Nousia* lineage) formed a monophyletic group with moderate bootstrap support. *Mauiulus* and *Austroclima* (*Dactylophlebia* lineage) were the sister-group to the clade comprising the genera of the *Meridialaris* lineage ('WT sp. 1', 'WT sp. 2', *Austrophlebioides*, *Meridialaris*, *Tillyardophlebia*, *Kirrara*, *Deleatidium* and *Atalophlebioides*). The monophyly of the *Meridialaris* clade was strongly supported, with a bootstrap value of 97%.

Discussion

The outcomes from the parsimony analysis are largely consistent with the higher-level relationships previously proposed by Pescador and Peters (1980). The only point of conflict between their phylogeny and the findings of the present study is the monophyly of the *Hapsiphlebia* lineage. Although the basal position of genera assigned to the *Hapsiphlebia* lineage (*Kalbaybaria*, *Atalophlebia*, *Atalomicria*, *Ulmerophlebia* and *Jappa*) was supported by the parsimony analysis, these genera did not form a monophyletic group. Two derived character states were given by Pescador and Peters (1980) in support for the monophyly of the

Hapsiphlebia lineage: (*i*) the presence of subapical denticles on the outer incisor of the right mandible (character 9); and (*ii*) prominent lateral setae on the abdominal terga of the nymph (character 29). In this study, a reduction or absence of ventral teeth on the tarsal claw of the nymph (character 26) was also observed among genera of the *Hapsiphlebia* lineage, but this feature is not unique to this group, having also occurred in *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* (Dean *et al*. 1999). Even though these possible synapomorphies were included in the present analysis, the

Table 1. Taxa included in the cladistic analysis and sources from which morphological characters were obtained

Taxon	Specimens examined	Literature sourced
Ingroup taxa		
Hapsiphlebia lineage		
Atalomicria sexfasciata	Australia: Qld. Cloudy Ck., 17.xi.1996 (N) Australia: Qld. Cloudy Ck., 29.iii.1998 (F) Australia: Qld. Cloudy Ck., 10.i.1999 (M)	Campbell and Peters (1993)
Atalophlebia sp. AV13	Australia: Qld. Birthday Ck., 22.ix.1996 (N) Australia: Qld. Birthday Ck., 25.xi.1996 (F) Australia: Qld. Camp Ck., 23.ix.1996 (M)	
Kalbaybaria doantrangae	Australia: Qld. Goolagan Ck., 25.viii.2000 (N)	Campbell (1993)
Ulmerophlebia sp. AV3	Australia: Qld. Birthday Ck., 22.ix.1996 (N) Australia: Qld. Camp Ck., 23.ix.1996 (F) Australia: Qld. Camp Ck., 24.x.1996 (M)	
Penaphlebia lineage		
Garinjuga sp.	Australia: Vic. O'Shannassy R., 4.i.1980 (N, M) Australia: Vic. Starvation Ck., 24.x.1975 (M)	Campbell and Suter (1988)
Nousia lineage		
Koorrnonga sp. AV6	Australia: Qld. Birthday Ck., 22.ix.1996 (N) Australia: Qld. Camp Ck., 23.ix.1996 (F, M)	Campbell and Suter (1988)
Nousia sp.	Australia: Qld. Cloudy Ck., 17.xi.1996 (N) Australia: Qld. Birthday Ck., 17.iv.1998 (F, M)	Campbell and Suter (1988)
Nyungara bunni	Australia: WA. Waterfall Gully, 16.ix.1981 (N) Australia: WA. Foster Br., 23.vii.1982 (M)	Dean (1987)
Dactylophlebia lineage		
Austroclima sepia	New Zealand: Kitekite Stm., 27.xii.1976 (N) New Zealand: Cascade Stm., 27.x.1975 (F)	Towns and Peters (1979)
Mauiulus luma	New Zealand: Cascade Stm., 23.i.1974 (N) New Zealand: Cascade Stm., 9.ii.1977 (F)	Towns and Peters (1979)
Meridialaris lineage		
Austrophlebioides pusillus	Australia: NSW. Mongarlowe R., 1.xii.1998 (N) Australia: NSW. Kangaroo R., 6.xii.1978 (F, M)	Campbell and Suter (1988)
Kirrara procera	Australia: NSW. Bolairo, 30.i.1966 (N)	Campbell and Peters (1986)
Tillyardophlebia rufosa	Australia: NSW. Pipers Ck., 11.ii.2000 (N)	Dean (1997)
'WT sp. 1'	Australia: Qld. Cloudy Ck., 17.xi.1996 (N) Australia: Qld. Cloudy Ck., 29.iii.1998 (F, M)	
'WT sp. 2'	Australia: Qld. Oliver Ck., 26.xii. 1997 (N) Australia: Qld. Oliver Ck., 23.viii. 1999 (F, M)	
Atalophlebioides cromwelli	New Zealand: Kapowai R., 14.i.1977 (N) New Zealand: Cascade Stm., 9.iii.1977 (F)	Towns and Peters (1978)
Deleatidium lillii	New Zealand: Pataruru, 16.v.1964 (N) New Zealand: Tyer's Stm., 24.i.1981 (F)	Towns and Peters (1996)
Meridialaris chiloeense	Chile: Puerto Ibanez, 12/17.i.1961 (N) Chile: El Canelo, 30.ix/2.xii.1972 (F) Chile: Rio Tupe, 19/21.xii.1972 (M)	Pescador and Peters (1987)
Affinities uncertain		
Bibulmena kadjina	Australia: WA. Seldom Seen Br., 17.ix.1981 (N)	Dean (1987)
Kaninga gwabbalitcha	Australia: WA. Carey Br., 15.xii.1989 (N)	Dean (2000)
Loamaggalangta sp.	Australia: Qld. Cloudy Ck., 29.iii.1998 (N) Australia: Qld. Cloudy Ck., 23/24.ix.1996 (F, M)	Dean *et al*. (1999)
Neboissophlebia hamulata	Australia: Vic. Starvation Ck., 19.xii.1979 (N)	Dean (1988)
Outgroup taxa		
Leptophlebia cupida	USA: Wisconsin, Sevenmile Ck., 7.v.1969 (N) Canada: Ontario, Kearney Ck., 26.v.1960 (F, M)	
Leptophlebia marginata		Peters and Edmunds (1970)

N, nymph; F, female imago; M, male imago.

Table 2. List of characters used in the cladistic analysis

Characters identified by Pescador and Peters (1980) and Towns and Peters (1980, 1996) as phylogenetically informative are indicated by an asterisk (*)

Nymph

Labrum/clypeus

*(*1*) *Labrum width/clypeus width:* (0) labrum narrower than clypeus; (1) labrum subequal or broader than clypeus.

Clypeus

*(*2*) *Lateral margins:* (0) sub-parallel or convergent apically; (1) slightly divergent apically; (2) divergent apically.

Labrum

*(*3*) *Anterior margin:* (0) straight or with broad emargination; (1) with narrow U or V-shaped emargination.
*(*4*) *Denticles on anterior margin:* (0) absent; (1) prominent; (2) greatly reduced.
(*5*) *Denticles:* (0) of similar length; (1) median denticle much longer.
*(*6*) *Median hood on anterior margin:* (0) absent; (1) present.
(*7*) *Setae on dorsal surface of labrum:* (0) scattered; (1) in 3 rows, apical, median and basal; (2) in 2 rows, apical and median; (3) in 1 or 2 rows, apical only or apical plus subapical.
(*8*) *Subapical (or median) setal row on dorsal surface of labrum:* (0) from three quarters to as wide as apical row; (1) up to half width of apical row; (2) wider than apical row.

Mandible

*(*9*) *Right outer incisor:* (0) slender, parallel-sided; (1) robust, triangular, with subapical denticles.
*(*10*) *Serrations on apex of outer incisors:* (0) absent; (1) present.
(*11*) *Tuft of setae midway along outer margin of mandible:* (0) absent; (1) present.
(*12*) *Series of long setae on basal 2/3 of outer margin:* (0) absent; (1) present.
(*13*) *Fine setae along apical 1/3 of outer margin between setal tuft and outer incisor:* (0) absent; (1) present.
*(*14*) *Prosthecal tuft:* (0) well developed; (1) greatly reduced.
*(*15*) *Prosthecal tuft:* (0) broad and fleshy at base; (1) slender and elongated or very reduced.

Hypopharynx

(*16*) *Lingua:* (0) without well developed lateral processes; (1) with well developed lateral processes.

Maxillae

*(*17*) *Galea-lacinia:* (0) narrow; (1) broad.
*(*18*) *Galea-lacinia:* (0) subapical pectinate setae absent; (1) with up to 15 subapical pectinate setae; (2) with 17 to 21 subapical pectinate setae; (3) usually with more than 22 subapical pectinate setae.
(*19*) *Outer margin of cardo fringed with:* (0) long or moderately long spines or setae; (1) short spines.

Labium

*(*20*) *Glossae:* (0) turned under ventrally, not on the same plane as paraglossae; (1) not turned under ventrally, on about the same plane as paraglossae.
*(*21*) *Submentum:* (0) with long spines along lateral margins; (1) bare or with few spines at base of lateral margins.
*(*22*) *Segment 3 of labial palp:* (0) 0.6 to 1.0 times length of segment 2; (1) less than 0.6 times length of segment 2; (2) greater than 2 times length of segment 2.
(*23*) *Labial palp length/width ratio of segment 3:* (0) < 1.5; (1) 1.6 to 2.1 (2) > 2.1.
(*24*) *Inner margin of segment 3 of labial palp:* (0) with stout spines; (1) with small denticles; (2) without spines or denticles.

Thorax

(*25*) *Long setae on lateral margins of pronotum:* (0) present; (1) absent.

Legs

(*26*) *Tarsal claws:* (0) with teeth; (1) with fine denticles; (2) smooth.
(*27*) *Spines on ventral margin of tarsi:* (0) all of similar length; (1) one or more apical spines greatly elongated on tarsi of all legs or on tarsi of mid and hind legs only.

Abdomen

(*28*) *Posterolateral projections on segments:* (0) 2 to 9; (1) 4, 5 or 6 to 9; (2) 7 or 8 to 9.
*(*29*) *Lateral margin of abdominal segments:* (0) bare; (1) fringed with fine setae; (2) fringed with spines and/or thick setae.
(*30*) *Row of setae on mid-dorsal region of segments:* (0) absent; (1) present.

Gills

(*31*) *Gill shape:* (0) plate-like to broadly ovate, apex rounded or with small point; (1) narrow to broadly ovate with single apical filament or lanceolate; (2) divided into 3 or more filaments.
(*32*) *Outer margin of gill:* (0) without dense fringe of setae; (1) with dense fringe of setae.

(*continued next page*)

Table 2. (continued)

Caudal filaments

(*33*) *Whorl of spines on segments:* (0) present; (1) absent.
(*34*) *Setae on segments:* (0) long; (1) short or of moderate length.

Adult

Hind wing

(*35*) *Length of subcostal vein:* (0) 0.9 or more of wing length; (1) less than 0.9 of wing length.
(*36*) *Total number of cross veins in hind wing:* (0) more than 18; (1) less than 15.

Legs

*(*37*) *Tarsal claws:* (0) similar, both hooked; (1) dissimilar, pad-like claw not hooked; (2) dissimilar, pad-like claw with small apical hook.

Female abdomen

*(*38*) *9th sternum with:* (0) deep to moderate cleft; (1) shallow cleft or entire.

Male eye

*(*39*) *Shape of facets of compound eye of adult male (as well as male subimago and male nymph):* (0) hexagonal; (1) square on upper portion of eye and hexagonal on lower portion.

Male genitalia

*(*40*) *Styliger plate:* (0) deeply cleft ventrally; (1) not deeply cleft ventrally, posterior ventral margin entire or with small U-shaped notch.
(*41*) *Penes:* (0) separated except at base; (1) separated in apical 1/2 to 1/5; (2) fused or separated only at very apex.
(*42*) *Apical half of penes with:* (0) spines absent or minute; (1) ventral spine or ventrally directed apical spine; (2) apical spine, spine directed apically; (3) dorsal spine, spine on accessory lobe; (4) dorsal spine, spine not on accessory lobe.
(*43*) *Midventral subapical appendage:* (0) absent; (1) present, appendage round and knob-like in ventral view; (2) present, appendage not round and knob-like in ventral view.

Hapsiphlebia lineage was not recovered as a monophyletic group, but instead appears to be a paraphyletic grade.

Relationships among the genera previously assigned to the *Hapsiphlebia* lineage were poorly supported, except for the sister-group relationship between *Jappa* and *Ulmerophlebia*. These two genera are closely related and morphologically very similar. The major difference between the nymphs of these genera is the presence of large frontal horns in *Jappa*. The many similarities of the nymphs and adults of the two genera have previously been discussed by Tsui and Peters (1975) and Suter (1986). Tsui and Peters (1975) also speculated that *Jappa* may have evolved from an *Ulmerophlebia*-like ancestor.

Kalbaybaria, placed as the sister-group to all other ingroup taxa, has many character states not shared by any of the other ingroup genera. The anterior margin of the labrum lacks denticles (character 4) and the setae on the dorsal surface are scattered rather than organised into distinct rows (character 7). Subapical pectinate setae are absent from the galea-lacinia of the maxilla (character 18), the second segment of the labial palp is elbow shaped and the third segment bears elongated setae. Campbell (1993) commented on the similarity of the nymphal gills and shape of the penes of *Kalbaybaria* to those of some species of *Atalophlebia*. However, these genera did not group together in the present study, instead *Atalophlebia* was placed as sister to *Atalomicria*, although this relationship was poorly supported.

The placement of *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* at the base of a large clade containing all other ingroup genera except those of the *Hapsiphlebia* grade was strongly supported, and is in agreement with discussions of the phylogenetic affinities of these genera by Dean (1987, 1988, 2000) and Dean *et al*. (1999). *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* differ from the genera of the *Hapsiphlebia* grade in the morphology of the nymphal mouthparts, especially the shape of the mandible. The nymphal mouthparts of these genera also display several features that are believed to be more derived than those found in the *Hapsiphlebia* grade. The labrum is subequal or broader than the clypeus (character 1), a median setal tuft is present along the outer margin of the mandible (character 11), the prosthecal tuft is elongated (character 15), and the inner margin of segment 3 of the labial palp has small denticles rather than stout spines (character 24). However, as discussed by Dean (1987, 1988, 2000) and Dean *et al*. (1999), these genera retain several ancestral character states that exclude them from other lineages, particularly the ventrally curved glossae of the labium (character 20) and the presence of denticles along the inner margin of segment 3 of the labial palp (character 24). They cannot be placed into the *Penaphlebia* lineage because they lack the defining pectinate setae on the second segment of the maxillary palp. *Neboissophlebia*, *Bibulmena*, *Loamaggalangta* and *Kaninga* appear to belong to lineages not previously recognised among Gondwanan Leptophlebiidae.

Garinjuga is the only known Australian genus of the *Penaphlebia* lineage, which also includes *Penaphlebia* and *Massartella* from South America (Pescador and Peters

1980). It was assigned to the *Penaphlebia* lineage by Campbell and Suter (1988) based on the possession of several derived character states, including the presence of pectinate setae on the second segment of the maxillary palp. In the present study, *Garinjuga* was placed as the sister-group to a large clade comprising genera of the *Nousia*, *Dactylophlebia* and *Meridialaris* lineages, although bootstrap support for this relationship was poor. In the previous cladistic analysis of Christidis (2001), *Garinjuga* was placed as either the sister-group to the *Nousia* lineage or sister to the *Meridialaris* lineage. However, in that analysis the *Dactylophlebia* lineage was not represented, and it appears that the inclusion of genera from the *Dactylophlebia* lineage in the present analysis may have aided in resolving the phylogenetic placement of *Garinjuga* (*Penaphlebia* lineage).

The grouping of *Nyungara*, *Nousia* and *Koorrnonga* into a monophyletic clade was well supported and is consistent with the current placement of these genera (Pescador and Peters 1980; Dean 1987; Campbell and Suter 1988). Derived character states given by Pescador and Peters (1980) for the lineage include the anterior margin of the nymphal labrum having a broad emargination (character 3) with prominent denticles (character 4). In the present study, a synapomorphic reduction in the number of cross veins of the hind wing (character 36) was observed in all genera and the length of the Sc vein ranged from 0.6 to 0.8 times the length of the hind wing (character 35). Relationships among *Nyungara*, *Nousia* and *Koorrnonga*, although resolved, were poorly supported.

As proposed by Pescador and Peters (1980), the *Dactylophlebia* lineage appears to be the sister-clade to the *Meridialaris* lineage. Features shared by the genera of these two groups include: labrum of the nymph with narrow emargination along anterior margin (character 3) and greatly reduced denticles (character 4); ninth abdominal sternum of adult female entire or with a shallow apical cleft (character 38); and dissimilar tarsal claws in the adult (character 37). The *Dactylophlebia* lineage has representative genera in southern South America and New Zealand but has not been recorded in Australia.

There was strong support for the monophyly of the *Meridialaris* lineage, comprising *Austrophlebioides*, *Tillyardophlebia*, *Kirrara*, 'WT sp. 1', and 'WT sp. 2' from Australia, *Meridialaris* from South America and *Deleatidium* and *Atalophlebioides* from New Zealand. These genera share several derived character states, all of which have been identified previously by Pescador and Peters (1980), as follows. In the nymph, the lateral margins of the submentum are bare or have only a few basal spines (character 21). The galea-lacinia of the maxilla is broad at the apex (character 17) and usually has more than 22 subapical pectinate setae (character 18), slightly fewer in *Kirrara*. The ante-

Table 3. Data matrix used in cladistic analysis

Taxa	Characters				
	0000000001	1111111112	2222222223	3333333334	444
	1234567890	1234567890	1234567890	1234567890	123
L. cupida	0000?00?00	0000000110	0010000220	1000002000	010
L. marginata	0000?00?00	0000000110	0010000200	1000002000	010
Atalophlebia	0001002010	0100010100	0000010020	2000100011	200
Atalomicria	0001002010	1100010100	0000020220	1000110011	210
Jappa	0001101010	0100010200	0011010221	1110101011	100
Ulmerophlebia	0001101010	0100010200	0011010121	1110101011	100
Neboissophlebia	1101001000	1000110200	0011020100	1000102011	010
Kalbaybaria	0000?00?10	?000010000	0220110220	2000000011	200
Bibulmena	1101003000	1000110200	0021120100	1000000011	200
Kaninga	1101003000	1000110310	0021110100	1001100011	000
Loamaggalangta	1101003000	1000110210	0021120200	1000100011	200
Garinjuga	1101003000	1100110201	0022100200	1001000011	040
Nyungara	1101003?00	1000110111	0022100100	1001110011	140
Nousia	1101003000	1000110111	0022100100	1001110011	100
Koorrnonga	1101003100	1000110111	0022100100	1001110011	100
Kirrara	1200?03?01	1000111211	1122101001	0001002111	001
Tillyardophlebia	1212013200	1010111311	1122100000	1001002111	000
'WT sp.1'	1112013200	1010111311	1122100000	1001102111	110
'WT sp.2'	1112013201	1010111311	1122100000	1001002111	140
Austrophlebioides	1212013201	1010111311	1122101010	1001002111	140
Meridialaris	1212013200	1010111311	1112101010	1001002111	220
Mauiulus	1112003000	1000110111	0022100200	1001102111	130
Austroclima	1112003000	1000110111	0022100200	1001102111	130
Deleatidium	1212013?01	1001111311	1122101000	0001001011	202
Atalophlebioides	1212013?01	1001111311	112210?000	1001?01111	202

?, Missing data or inapplicable character.

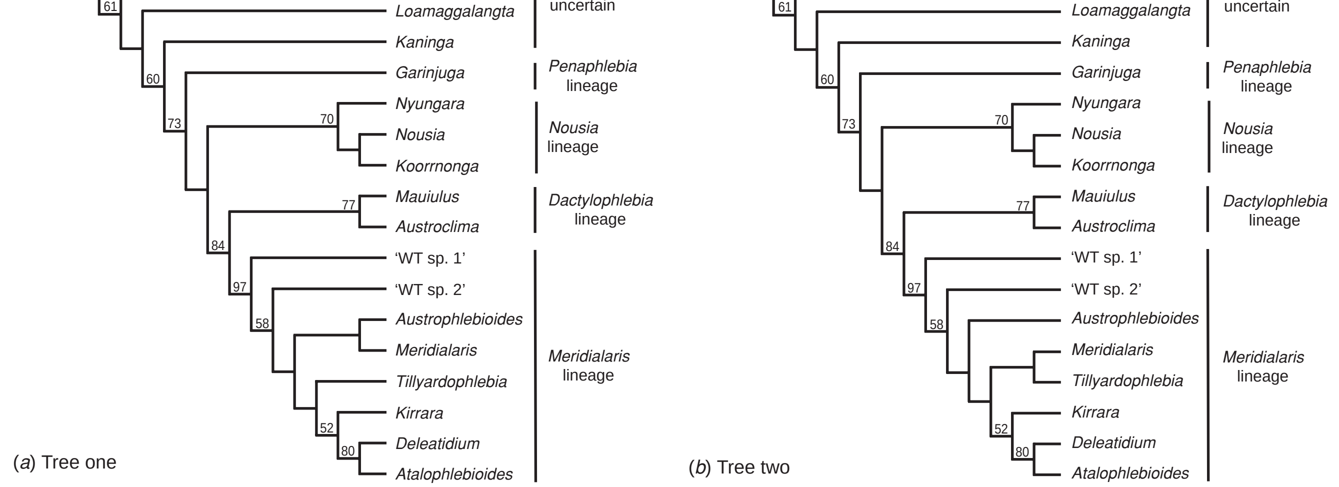
rior margin of the labrum has a narrow U- or V-shaped emargination (character 3) that is hooded dorsally (character 6) in all genera except *Kirrara*. It appears that this emargination may have been secondarily lost in *Kirrara* (Campbell and Peters 1986). In the adult, the tarsal claws are dissimilar (character 37) and the ninth abdominal sternum of the female is entire or has a very shallow cleft (character 38), except in some species of *Deleatidium* that have a more pronounced cleft (Towns and Peters 1996). Dissimilar claws are not unique to this group but appear to have evolved independently several times within the Leptophlebiidae, including *Jappa*, *Ulmerophlebia* and *Neboissophlebia*.

Two additional derived character states given by Pescador and Peters (1980) for the *Meridialaris* lineage are: (*i*) the outer margin of the mandible distinctly angular; and (*ii*) labrum with a length width ratio of 1/3 to 1/2. The outer margin of the mandible is very angular in *Kirrara* and *Deleatidium*, but is variable among the other genera. The shape of the outer margin was a difficult character to code into discrete states and was not included in this study. Similarly, labrum shape was variable within this lineage and a labrum with a length/width ratio of 1/3 to 1/2 was not unique to this group.

The two undescribed taxa, ‘WT sp. 1’ and ‘WT sp. 2’, clearly belong to the *Meridialaris* lineage. The nymphs of these two taxa share some similarities with those of *Tillyardophlebia* but the genitalia of the adult males differ in shape. The penes of the two undescribed taxa lack the diagnostic pair of ventral spines present at the base of the penes of *Tillyardophlebia* and are not divided but fused along most of their length. It is likely that these taxa represent two new genera.

Kirrara appeared to have a closer relationship to *Deleatidium* and *Atalophlebioides* of New Zealand than to the other Australian genera within this lineage, although support for this clade was low. The grouping of *Deleatidium* and *Atalophlebioides* is consistent with Towns and Peters (1978, 1996) suggestion that *Atalophlebioides* is most closely related to *Deleatidium*. The penes of these two genera are both fused (character 41) and have a midventral subapical appendage (character 43). The tarsal claws of the adult are dissimilar, as in other genera of the lineage; however, in *Deleatidium* and *Atalophlebioides*, the pad-like claw has a small apical hook (character 37) not found in the other genera. In the nymph, the prosthecal tuft of the mandible is greatly reduced in both genera (character 14).

Relationships among *Tillyardophlebia*, *Austrophlebioides, Meridialaris* and the clade containing *Kirrara*, *Deleatidium* and *Atalophlebioides* could not be resolved in the strict consensus tree. This was partly a result of homoplasy among several characters. For example, the outer incisors of the mandibles (character 9) are serrated in



(*a*) Tree one (*b*) Tree two

Fig. 2. Two most parsimonious trees obtained from a branch-and-bound search. Bootstrap values are shown above the nodes for those groups with > 50% support.

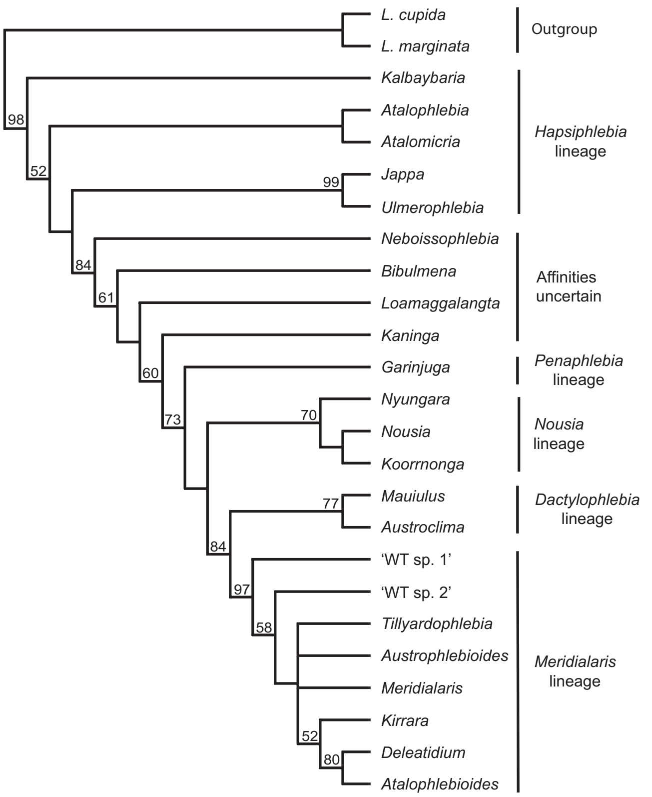


Fig. 3. Strict consensus tree of two most parsimonious trees obtained from a branch-and-bound search. Bootstrap values are shown above the nodes for those groups with > 50% support.

Deleatidium, *Atalophlebioides, Kirrara*, *Austrophlebioides* and 'WT sp. 2', but not serrated in *Meridialaris, Tillyardophlebia* and 'WT sp. 1'. This implies that serrations may have evolved independently on several occasions or have been secondarily lost in some genera. Likewise, the nymphs of many of the genera of the *Meridiararis* lineage have one or two elongated spines along the inner margin of the tarsi (character 27), exceptions being *Tillyardophlebia*, 'WT sp. 1' and 'WT sp. 2'. Again, these elongated spines may have evolved on more than one occasion or may have been secondarily reduced or lost. Clearly, additional data (morphological and molecular) will be required to resolve relationships within the *Meridialaris* lineage.

Acknowledgments

I thank Peter Cranston, John Dean and Richard Pearson for helpful comments on an earlier version of the manuscript. I am very grateful to John Dean (EPA Victoria), Terry Houston (Western Australian Museum), Bill Peters (Florida A&M University, USA), Trevor Crosby (Land Care, New Zealand) and David Goodger (Natural History Museum, England) for making available some of the specimens used in this study. This research was supported by funding from Land and Water Australia and James Cook University.

References

Campbell, I. C. (1993). A new genus and species of leptophlebiid mayfly (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) from tropical Australia. *Aquatic Insects* **15**, 159–167.

Campbell, I. C., and Peters, W. L. (1986). Redefinition of *Kirrara* Harker with a redescription of *Kirrara procera* Harker (Ephemeroptera: Leptophlebiidae: Atalophlebiinae). *Aquatic Insects* **8**, 71–81.

Campbell, I. C., and Peters, W. L. (1993). A revision of the Australian Ephemeroptera genus *Atalomicria* Harker (Leptophlebiidae: Atalophlebiinae). *Aquatic Insects* **15**, 89–107.

Campbell, I. C., and Suter, P. J. (1988). Three new genera, a new subgenus and a new species of Leptophlebiidae (Ephemeroptera) from Australia. *Journal of the Australian Entomological Society* **27**, 259–273.

Christidis, F. (2001). A cladistic analysis of *Austrophlebioides* and related genera (Leptophlebiidae: Atalophlebiinae). In 'Trends in Research in Ephemeroptera and Plecoptera'. (Ed. E. Dominguez.) pp. 305–312. (Kluwer Academic/Plenum Publishers: New York, USA.)

Dean, J. C. (1987). Two new genera of Leptophlebiidae (Insecta: Ephemeroptera) from south-western Australia. *Memoirs of the Museum of Victoria* **48**, 91–100.

Dean, J. C. (1988). Description of a new genus of leptophlebiid mayfly from Australia (Ephemeroptera: Leptophlebiidae: Atalophlebiinae). *Proceedings of the Royal Society of Victoria* **100**, 39–45.

Dean, J. C. (1997). Descriptions of new Leptophlebiidae (Insecta: Ephemeroptera) from Australia. I. *Tillyardophlebia* gen. nov. *Memoirs of the Museum of Victoria* **56**, 83–89.

Dean, J. C. (2000). Descriptions of new Leptophlebiidae (Insecta: Ephemeroptera) from Australia. II. *Kaninga*, a new monotypic genus from south-western Australia. *Records of the Western Australian Museum* **20**, 87–94.

Dean, J. C., Forteath, G. N. R., and Osborn, A. W. (1999). *Loamaggalangta pedderensis* gen. & sp. nov.: a new mayfly from Tasmania (Ephemeroptera: Leptophlebiidae: Atalophlebiinae). *Australian Journal of Entomology* **38**, 72–76. doi:10.1046/j.1440-6055.1999.00086.x

Edmunds, G. F., Jr, Jensen, S. L., and Berner, L. (1976). 'The Mayflies of North and Central America.' (University of Minnesota Press: Minneapolis, MN, USA.)

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Hillis, D. M., and Bull, J. J. (1993). An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Systematic Biology* **42**, 182–192.

Pescador, M. L., and Peters, W. L. (1980). Phylogenetic relationships and zoogeography of cool-adapted Leptophlebiidae (Ephemeroptera) in Southern South America. In 'Advances in Ephemeroptera Biology'. (Eds J. F. Flannagan and K. E. Marshall.) pp. 43–56. (Plenum Press: New York, USA.)

Pescador, M. L., and Peters, W. L. (1987). Revision of the genera *Meridialaris* and *Massartellopsis* (Ephemeroptera: Leptophlebiidae: Atalophlebiinae) from South America. *Transactions of the American Entomological Society* **112**, 147–189.

Peters, W. L., and Edmunds, G. F., Jr (1970). Revision of the generic classification of the Eastern Hemisphere Leptophlebiidae (Ephemeroptera) *Pacific Insects* **12**, 157–240.

Peters, W. L., and Edmunds, G. F., Jr (1972). A revision of the generic classification of certain Leptophlebiidae from southern South America (Ephemeroptera). *Annals of the Entomological Society of America* **65**, 1398–1414.

Suter, P. J. (1986). The Ephemeroptera (Mayflies) of South Australia. *Records of the South Australian Museum* **19**, 339–397.

Swofford, D. L. (2002). ‘PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods) Version 4.0b10.’ (Sinauer Associates: Sunderland, MA, USA.)

Towns, D. R., and Peters, W. L. (1978). A revision of the genus *Atalophlebioides* (Ephemeroptera: Leptophlebiidae). *New Zealand Journal of Zoology* **5**, 607–614.

Towns, D. R., and Peters, W. L. (1979). Three new genera of Leptophlebiidae (Ephemeroptera) from New Zealand. *New Zealand Journal of Zoology* **6**, 213–235.

Towns, D. R., and Peters, W. L. (1980). Phylogenetic relationships of the Leptophlebiidae of New Zealand (Ephemeroptera). In ‘Advances in Ephemeroptera Biology’. (Eds J. F. Flannagan and K. E. Marshall.) pp. 57–69. (Plenum Press: New York, USA.)

Towns, D. R., and Peters, W. L. (1996). ‘Fauna of New Zealand Volume 36. Leptophlebiidae (Insecta: Ephemeroptera).’ (Manaaki Whenua Press: Lincoln, New Zealand.)

Tsui, P. T. P., and Peters, W. L. (1975). The comparative morphology and phylogeny of certain Gondwanian Leptophlebiidae based on the thorax, tentorium, and abdominal terga (Ephemeroptera). *Transactions of the American Entomological Society* **101**, 505–595.

Manuscript received 30 May 2005, revised and accepted 24 October 2005.